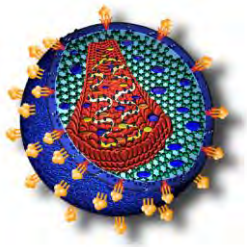

HIV Drug Resistance Program

National Cancer Institute at Frederick

The Challenge of an HIV Cure

HIV provirus integration and expression on long-term suppressive therapy

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Tufts University

Tufts UNIVERSITY

DEPARTMENT OF HEALTH & HUMAN SERVICES · USA

Objectives

1. To understand the role of HIV in causing AIDS

2. To understand how antiretroviral drugs control, but do not cure HIV infection

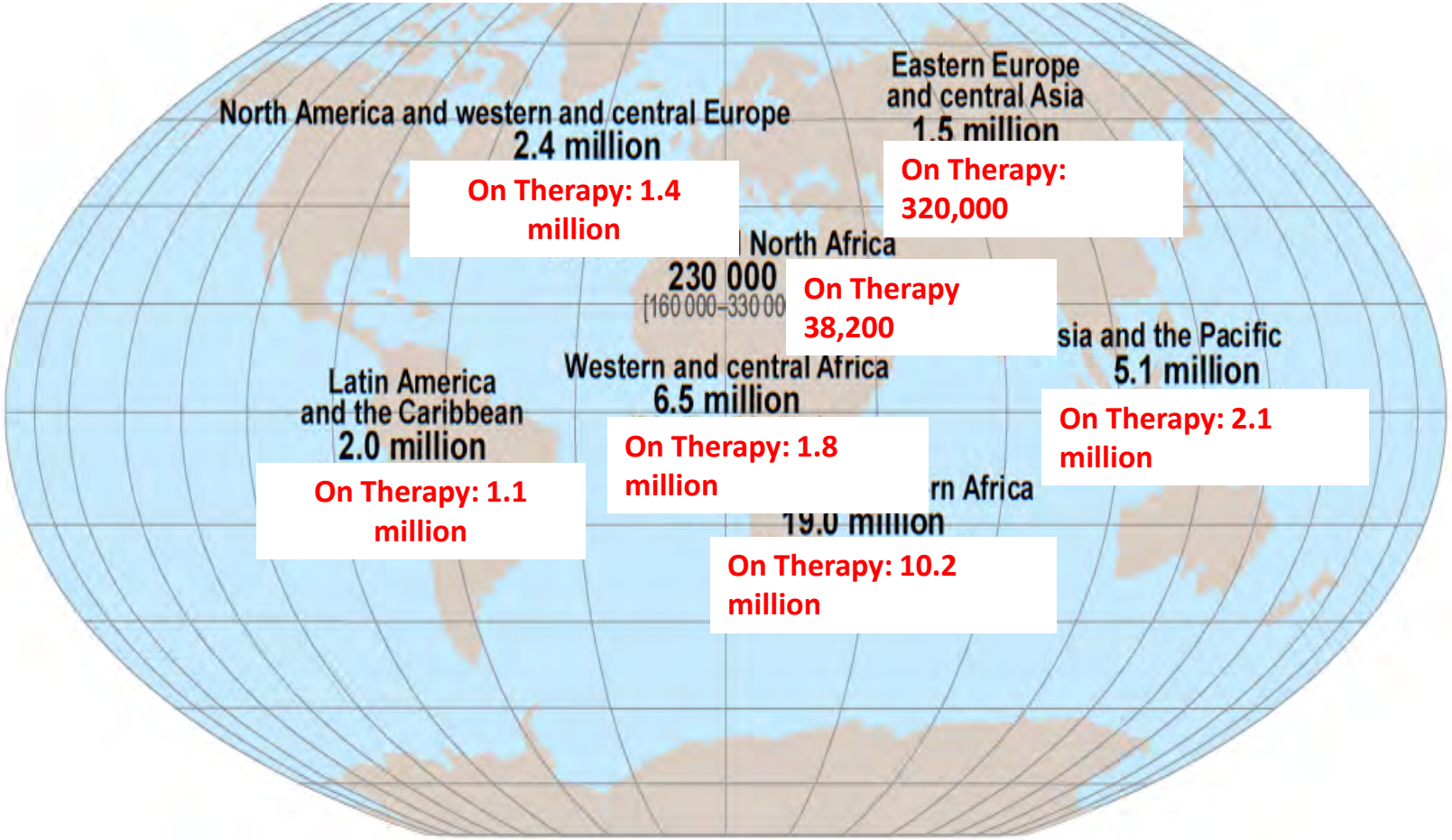
3. To understand the role of persistently infected, dividing, cells in HIV persistence

Disclosures

1. Financial:
   Tocagen, Inc. SAB member and shareholder

2. Off-label or unapproved drug recommendations:
   None

Global Burden of HIV Infection



Total: 36.7 million [34.0-39.8 million]

People undergoing antiretroviral therapy: 17 million

WHO, 2015

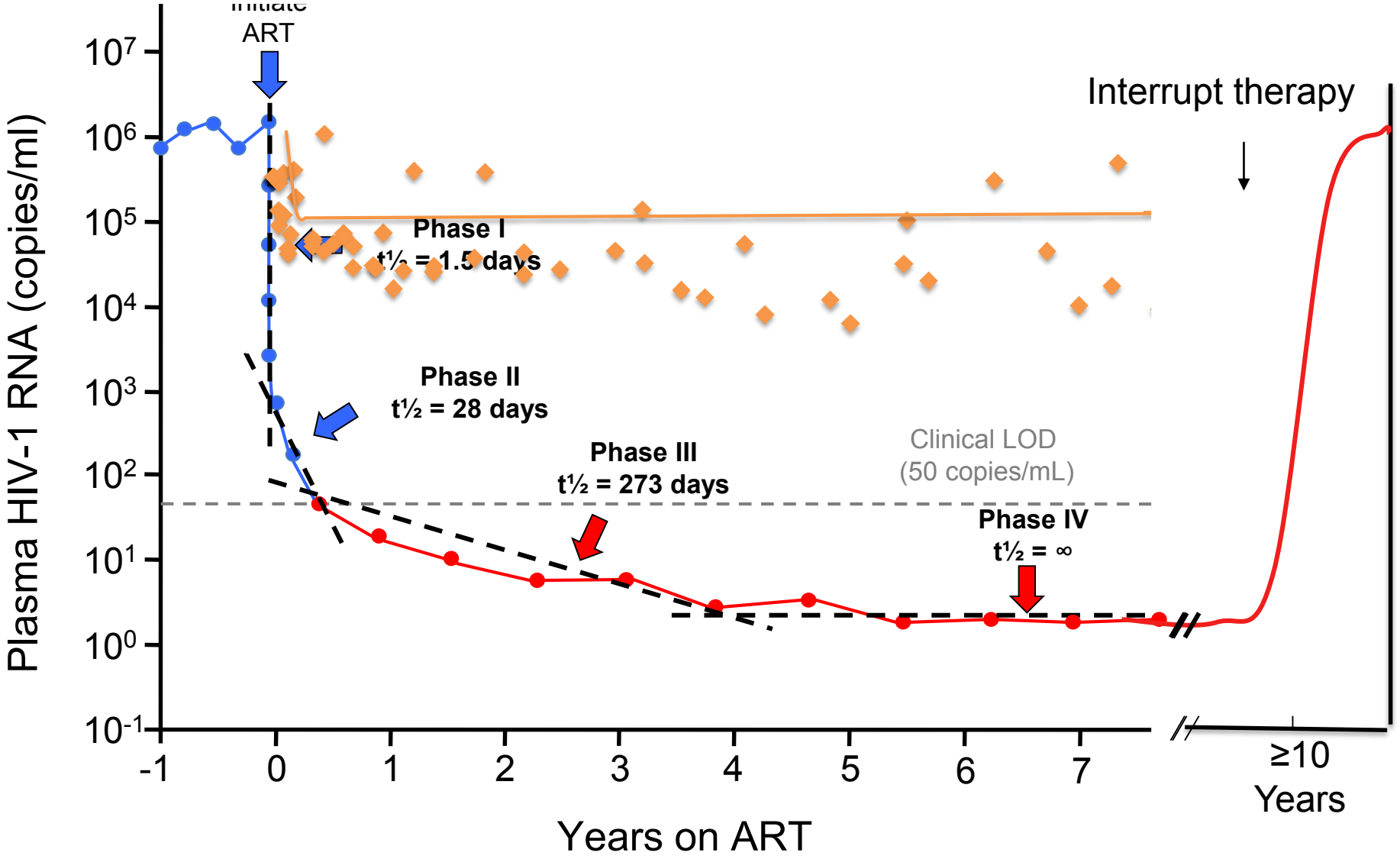
Challenges of HIV Infection

- Current antiviral therapies that can fully suppress viral replication and allow infected people to live a mostly normal life, but there are still both individual andand global challenges.
- Only about half the affected population has access to drugs, particularly in the worst-hit areas.
- Effective means of blocking transmission (PREP) are known, but not widely used. A vaccine is still a dream.
- Development of resistance to antiviral drugs is still a major issue, particularly in poorly-resourced areas.
- Antiviral drugs effectively suppress HIV infection, but will never cure it.

Timothy Brown was cured with a bone marrow transplant.

Why can’t we cure HIV Infection in everyone?

HIV-1 DNA Decays Much Less Than Plasma Virus RNA after Initiating ART

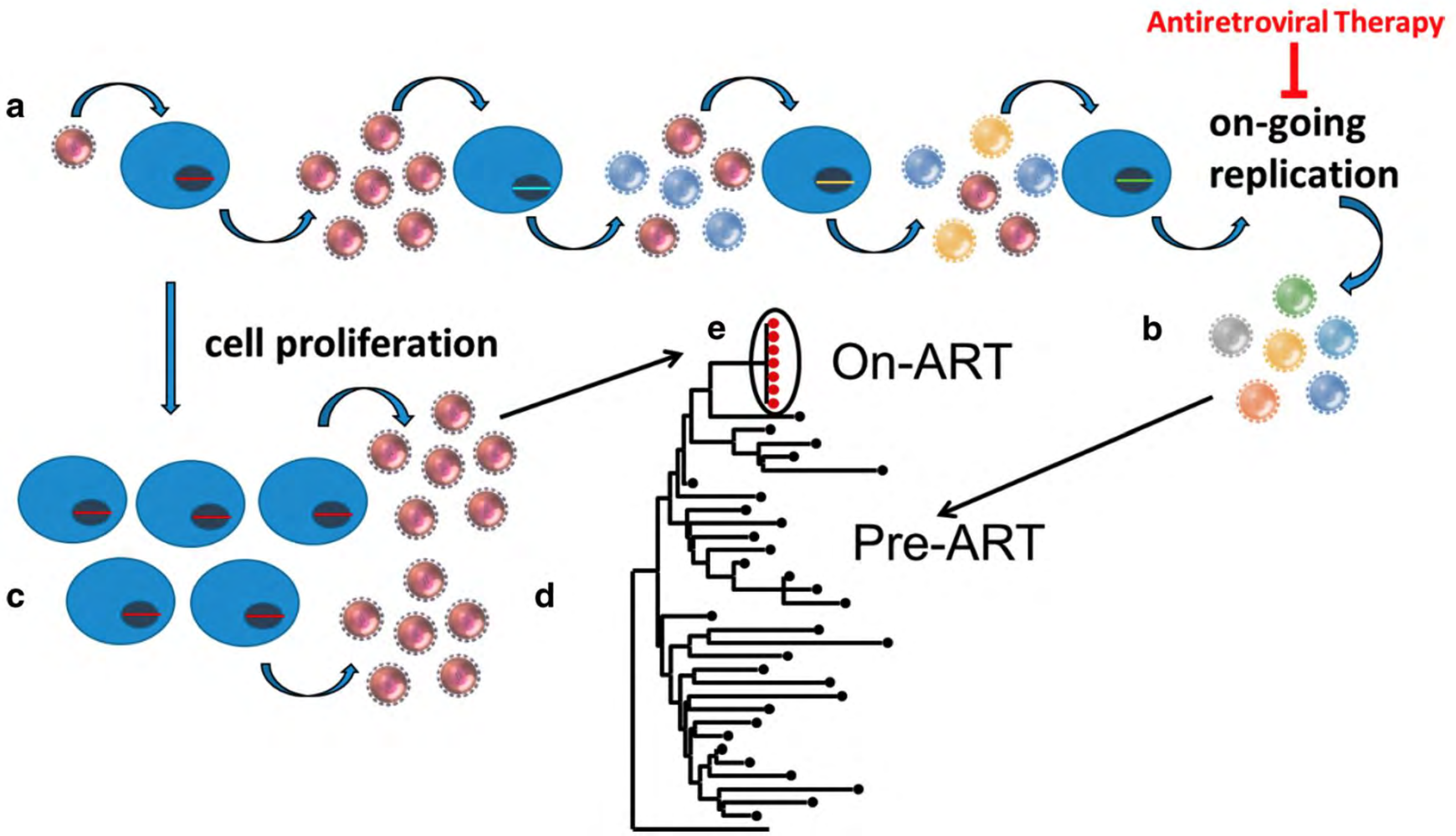


Courtesy Ben Hilldorfer, UPitt

Two Features of HIV Biology are Helpful in Understanding Persistence

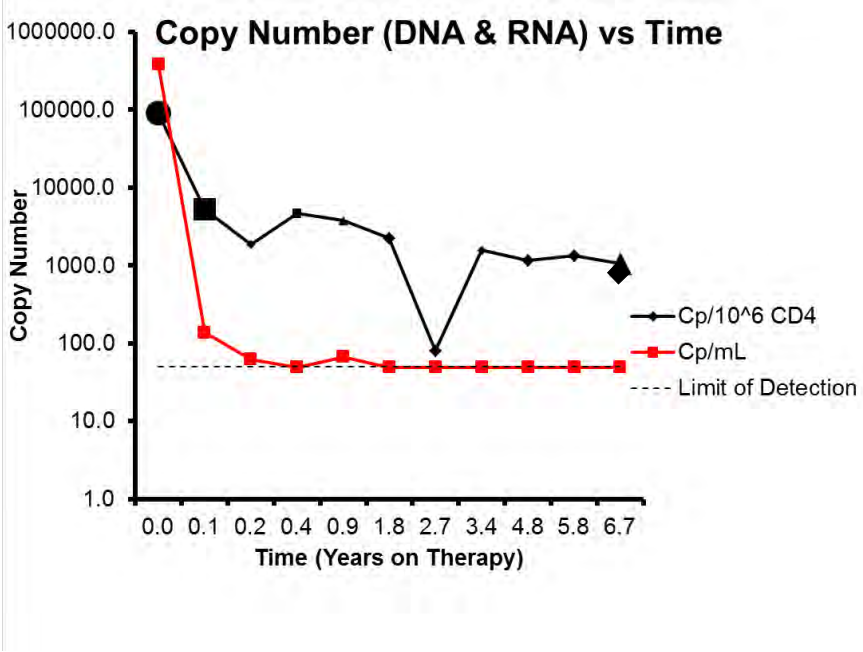
- Generation of genetic diversity during ongoing virus replication.
- Stable integration of DNA copy (provirus) of the viral genome at one of millions of sites in the host cell DNA
- The first important point is to distinguish between two models: ongoing low-level replication and latent proviruses in long-lived cells.

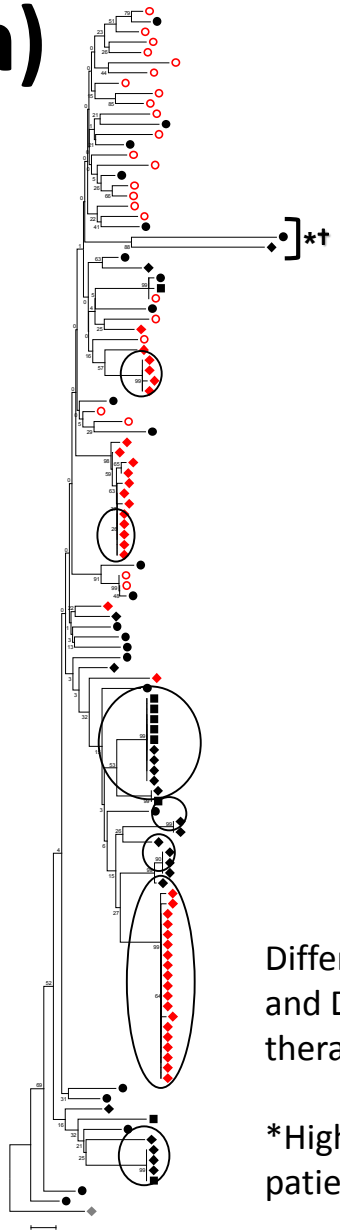
Inferring Virus Replication from Genetic Diversity



van Zyl et al Retrovirology 2018

Clonal Expansion of RNA and DNA Sequences During Suppressive ART (NO Evidence for Evolution)



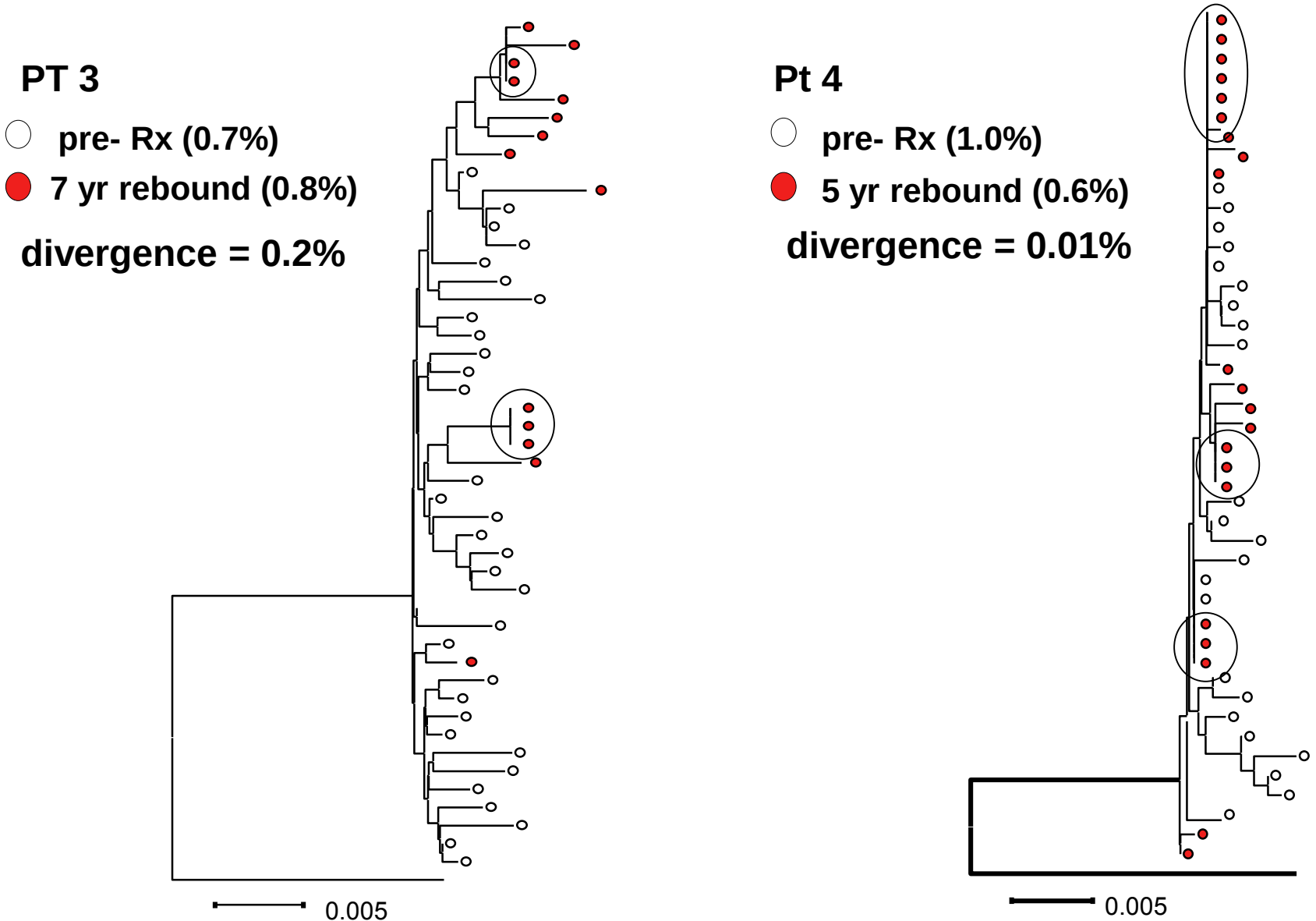


- 3 weeks
6 weeks
2.7 years
6.7 years
7.2 years
Reference
*G>A hypermutant
†stop codon

Different clonal populations of RNA and DNA appear after years of therapy

*Highly variable from patient to patient

No Evolution from Pre-therapy in Rebound Viremia After Long-term ART

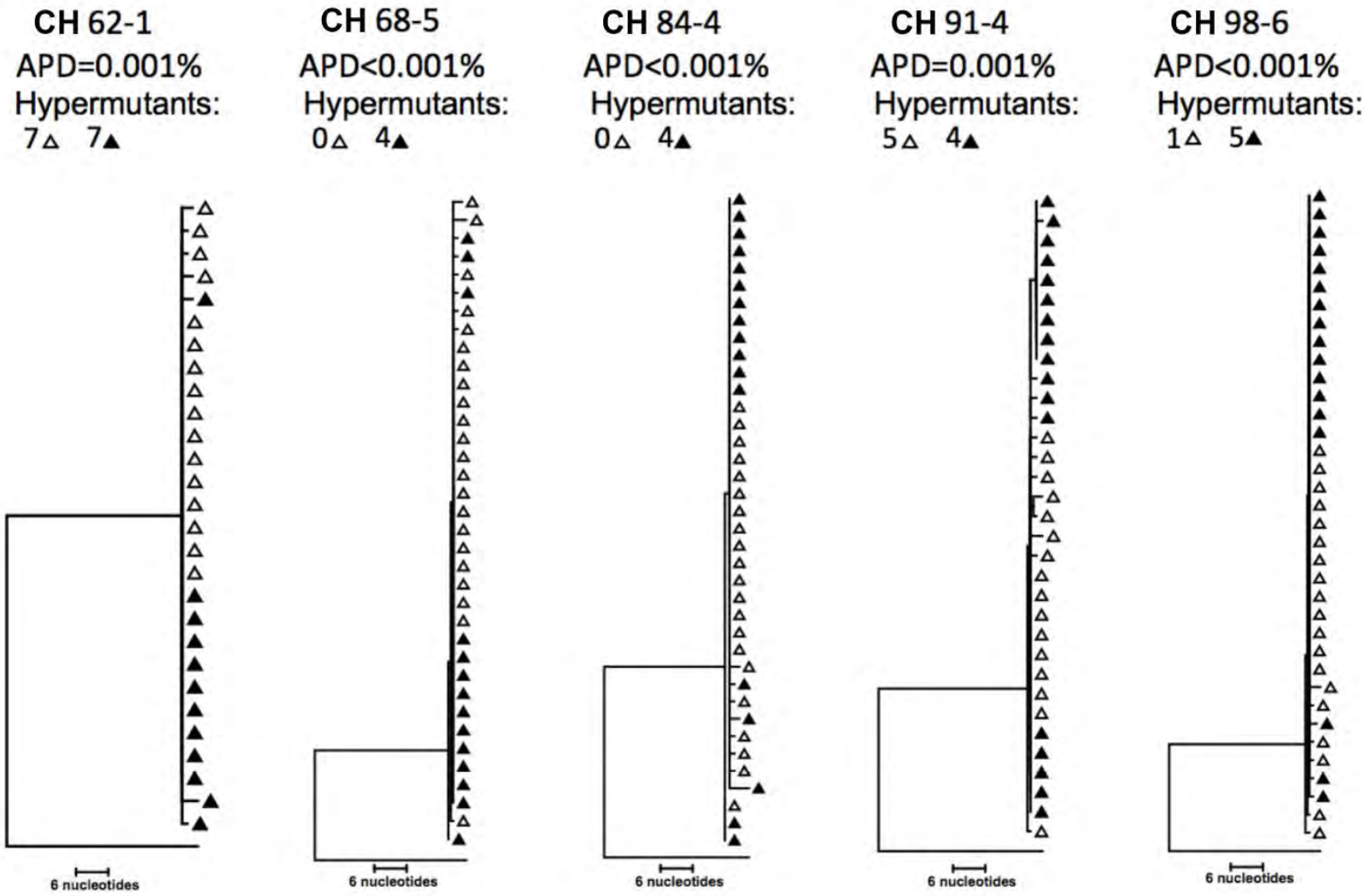


Is There Ongoing HIV Replication on ART?

- The problem with the previous studies was that the high diversity of HIV in chronically-infected individuals made it difficult to detect additional diversification on ART.
- Therefore we studied HIV populations in a set of infected people who were diagnosed and started on antiretroviral therapy (ART) within a few weeks of infection, when the virus population has had very little time to evolve.
- Although very difficult to identify, these patients provide a much stronger signal to detect HIV evolution.

No Evidence for Ongoing HIV Replication on ART?

(At least in blood)



Summary and Implications

- No evidence for on-going cycles of viral replication in individuals fully suppressed on ART
- Implies that the HIV reservoir is not maintained by on-going cycles of viral replication and therefore developing more potent ART will not cure HIV infection
- Others have proposed that ongoing replication during ART is not seen in blood, but does occur in the lymphoid compartment

- What's going on in lymph nodes?

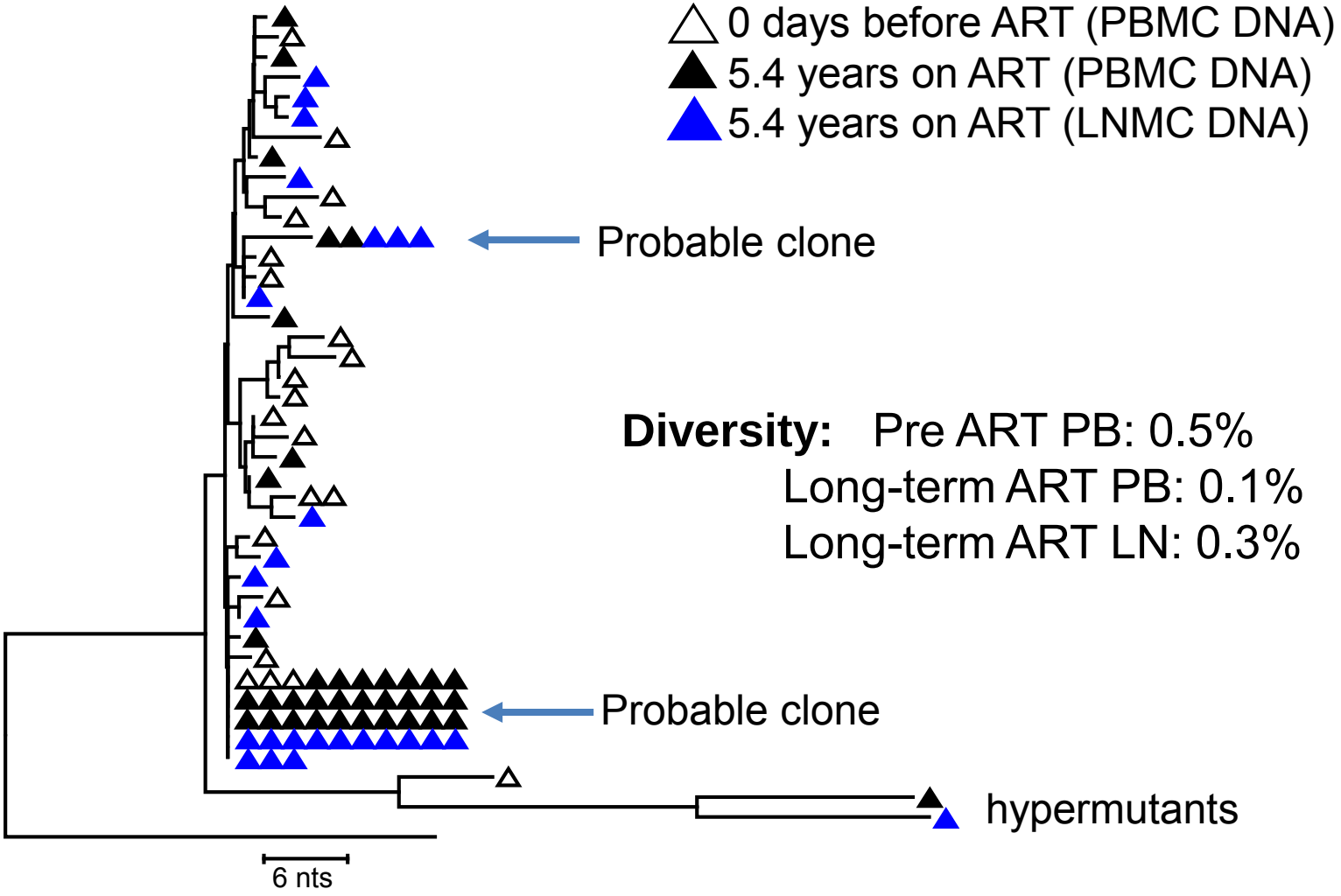
Does HIV Replication Persist in Lymphoid Tissues During ART?

- Sequenced paired lymph node and PBMC samples in four suppressed individuals and compared to pre-ART populations
- Sequenced longitudinal lymph node samples from individuals on ART

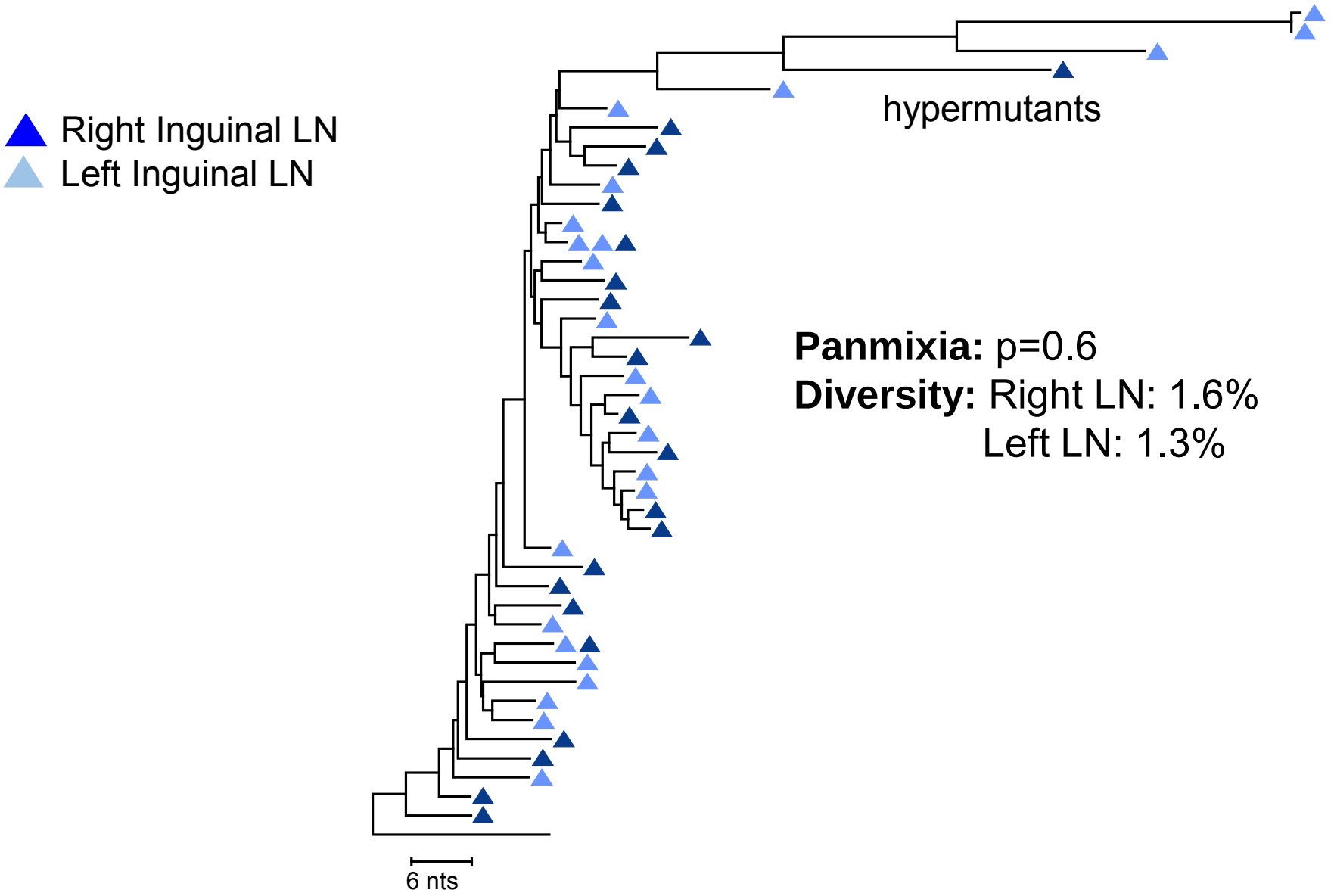
Delaney AIDS Research Enterprise
DARE
to find a cure

UCSF

Proviral Populations Are Not Different in PB and LN After 5 Years of Suppression on ART



No Difference in HIV Populations Across Lymph Nodes



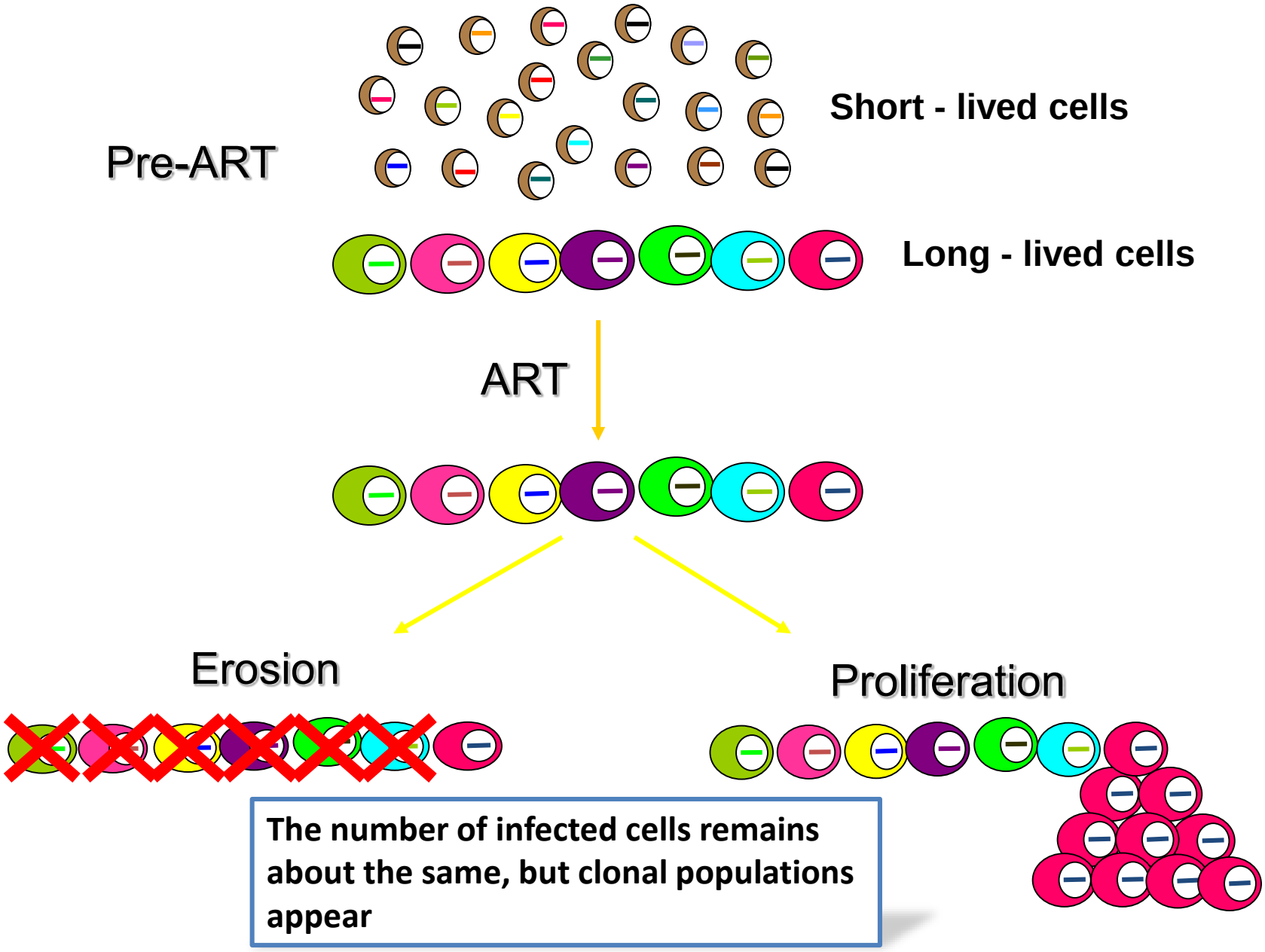
Summary and Implications

- No evidence for on-going cycles of viral replication in lymph nodes of adults fully suppressed on ART
- No evidence for “compartmentalization” of infected cells among lymph nodes and peripheral blood
- The HIV reservoir is not maintained by on-going cycles of viral replication and therefore developing more potent ART will not cure HIV infection
- Are identical sequences expanded clones or are they identical HIV variants (founder virus maybe?) with different integration sites?

NO Evidence for a Role of HIV Replication in Maintaining the True Reservoir

What does maintain the reservoir?

The Persistent Steady State



Integration Site Preferences 1.

- HIV DNA can irreversibly integrate at many millions of possible sites in the cell gnome, and sites of integration can uniquely "tag" single infected cells and their progeny.
- In long-lived HIV-infected cells, sites of integration are determined both by initial preferences (i.e.., "hot spots"), by selection after integration, and by chance.
- The assay we used (thanks to Rick Bushman and Charles Bangham) involves shearing infected cell DNA, ligating linkers, PCR amplification with LTR and linker-specific primers, and paired-end Illumina sequencing.
- Integration site is adjacent to LTR primer, and breakpoint is net to linker-specific primer.
- Multiple sequences with the Identical integration site and multiple breakpoints imply clonal expansion of the cell after infection.

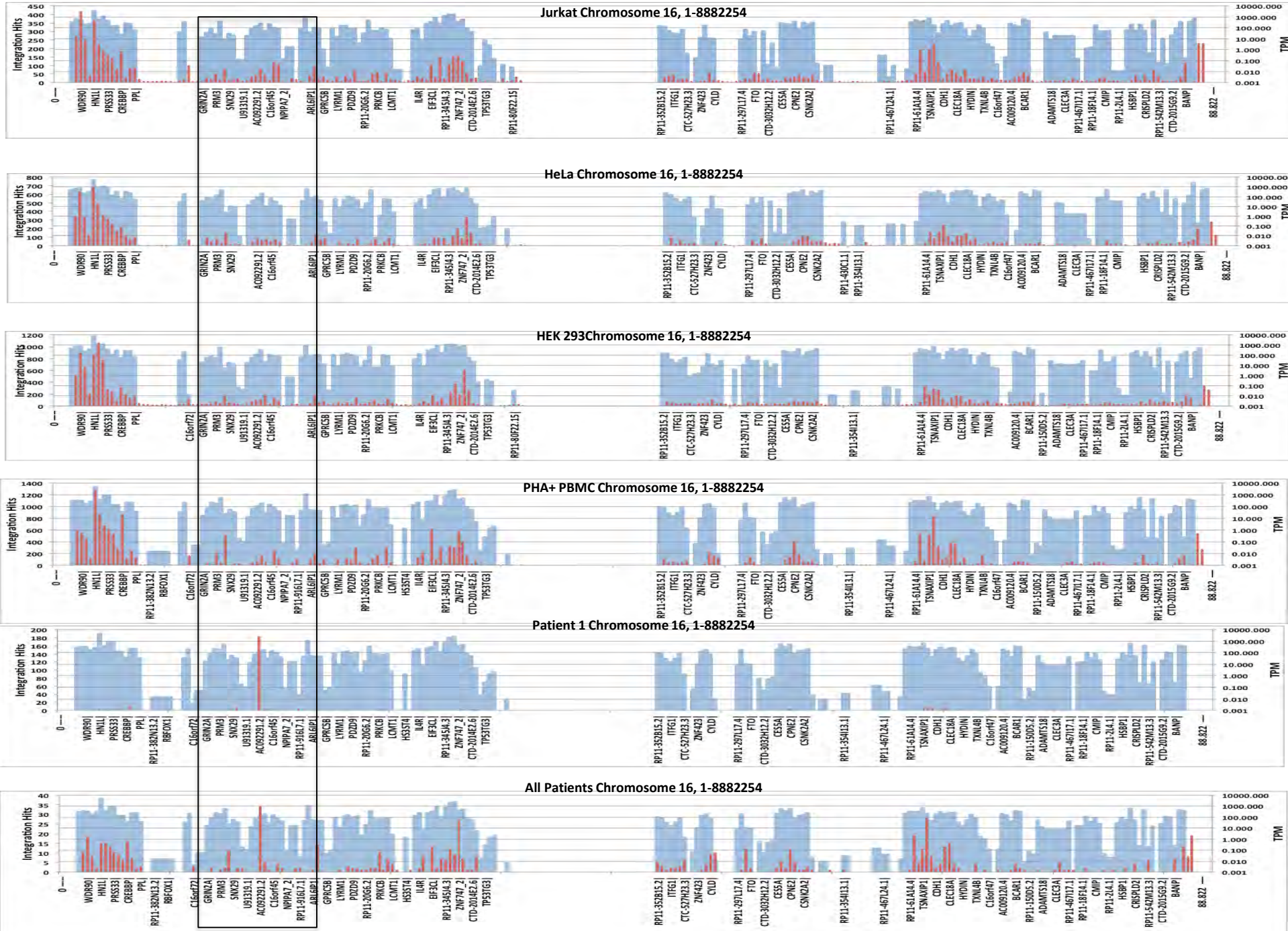
Multi-Scale Analysis of HIV Integration *in* and *ex Vivo*

- We have assessed integration site distribution in cultured cells (PBMC, stem cells, various cell lines (>150,000 sites each) or in HIV infected patients during suppressive ART, and compared with gene expression (RNA-seq).

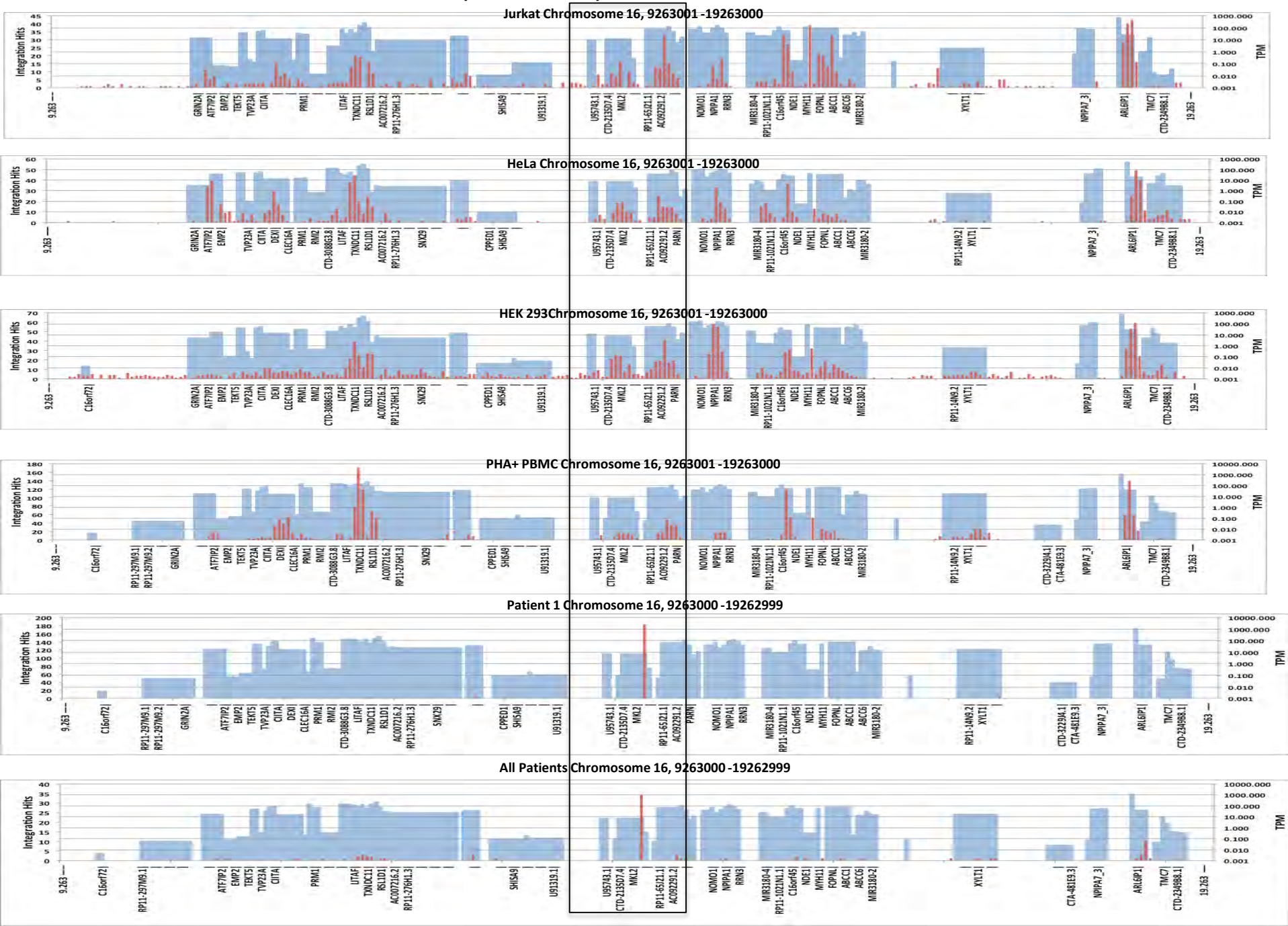
- In the next slides, cumulative integration sites in each interval of chromosome 16 are shown in red, and the expression level of each gene or region in blue.

- As you will see, the distribution of integration sites is highly similar, even between freshly isolated PBMCs and highly aneuploid epithelial cell lines, like HeLa.

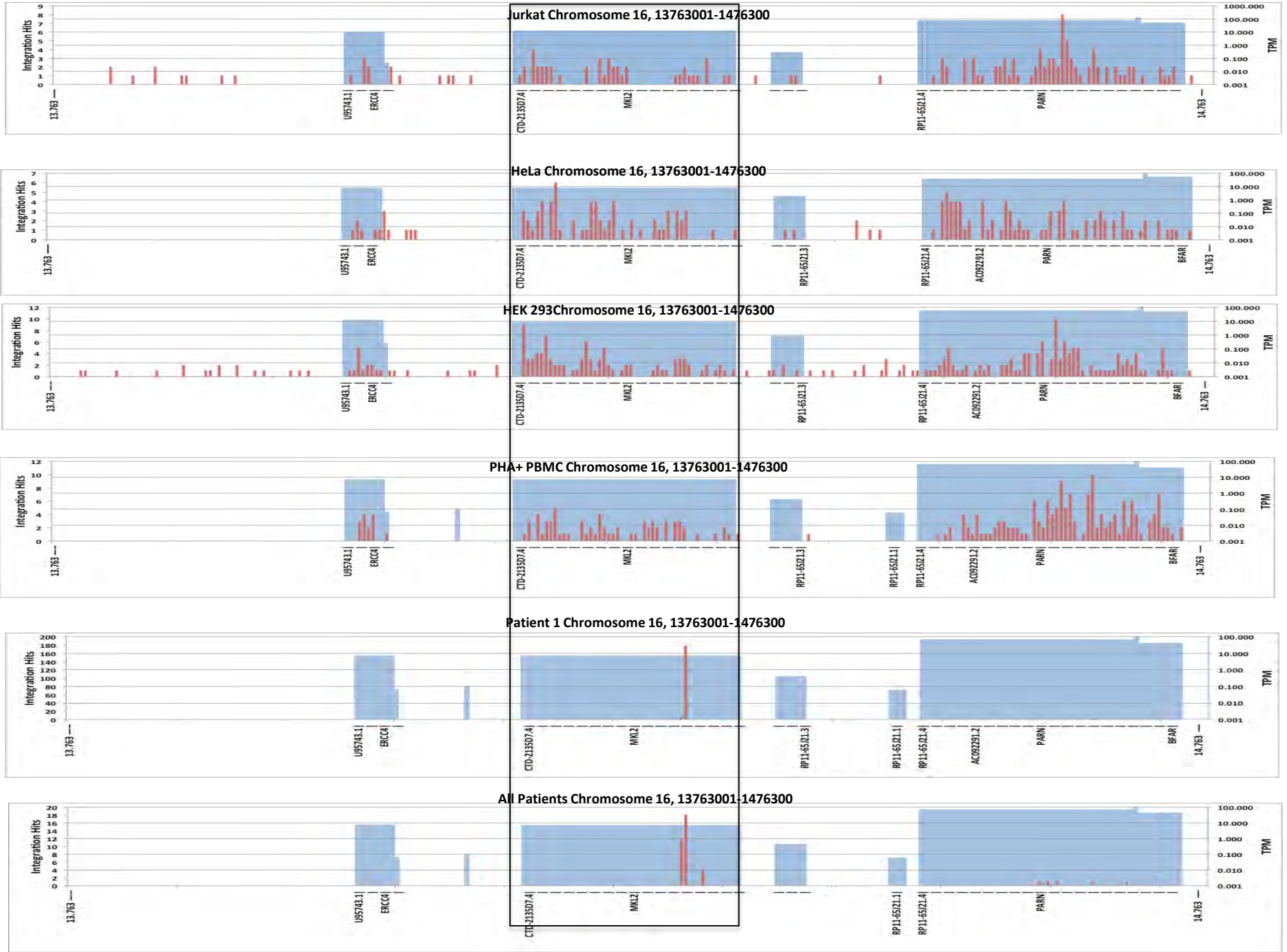
A. Whole Chromosome 16 (350 kb/bin)



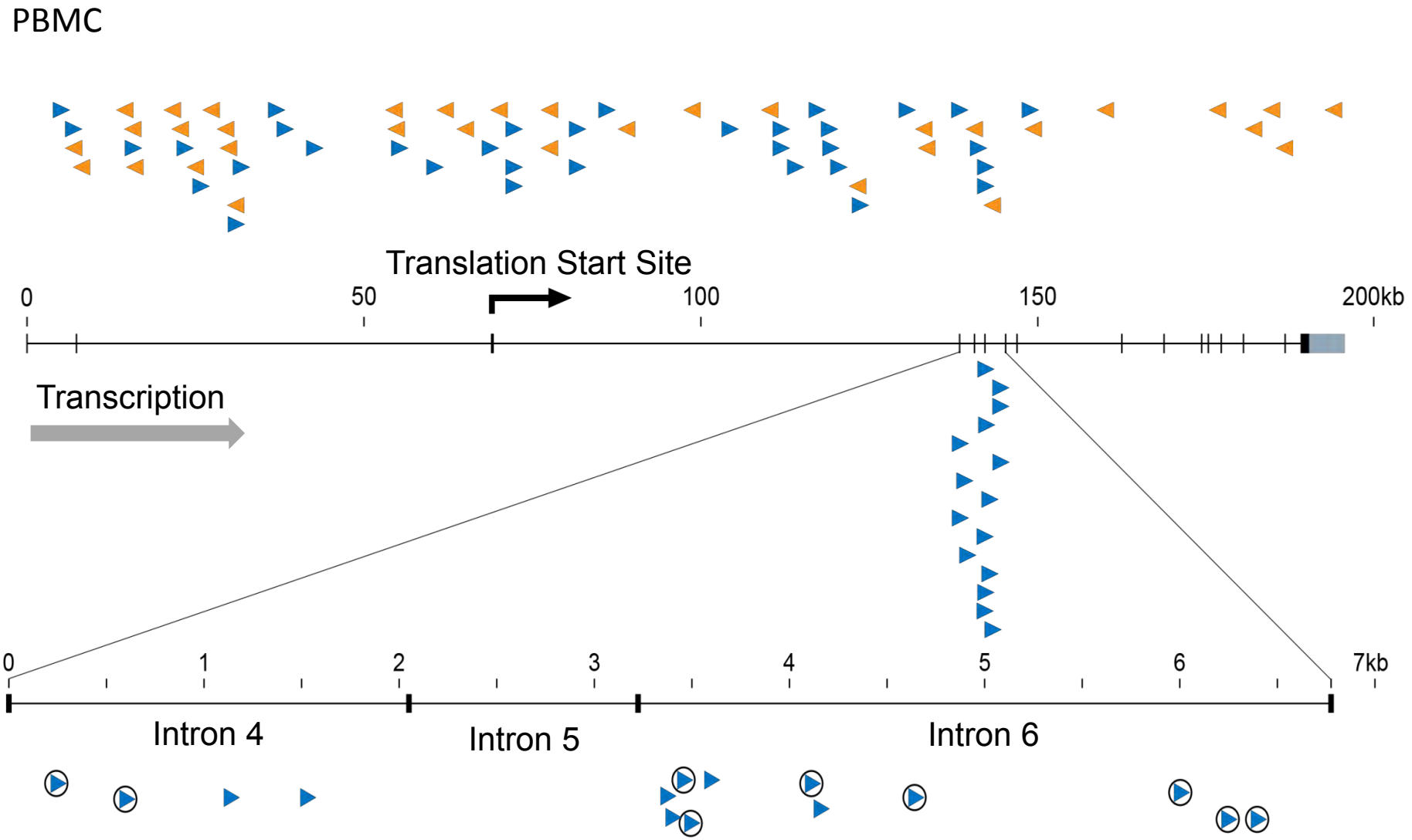
B. Chromosome 16 ca 10X from A (40 kb/bin)



C. Chromosome 16 10X from B (4kb/bin)



Integration Sites in MKL2 in Patient 1



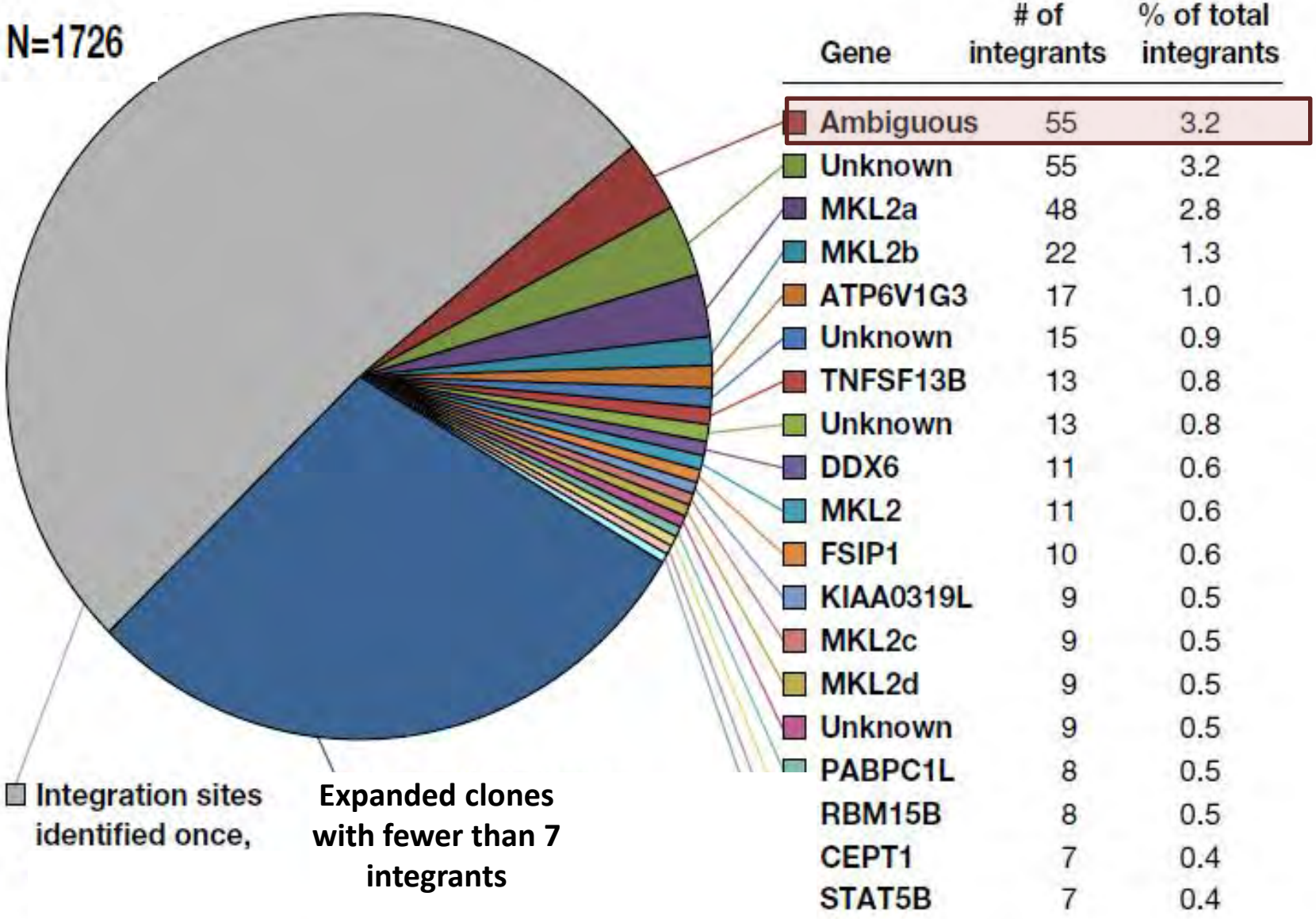
Integration Site Preferences 2. Selection for Specific Regions

- In long-lived HIV-infected cells, sites of integration are determined both by initial preferences (i.e.., “hot spots”) and by selection after integration.

- Integrations in MKL2 (and a couple of other genes) in patient 1 are clearly due to selection for preferential growth or survival due to effects of provirus on host gene expression, reminiscent of well-known models of retroviral oncogenesis.

- Only a very small fraction of proviruses seem to be involved in such effects. What does the overall distribution look like?

Integration Site Analysis Identifies Highly Expanded Clones in Patient 1



Gene	# of integrants	% of total integrants
Ambiguous	55	3.2
Unknown	55	3.2
MKL2a	48	2.8
MKL2b	22	1.3
ATP6V1G3	17	1.0
Unknown	15	0.9
TNFSF13B	13	0.8
Unknown	13	0.8
DDX6	11	0.6
MKL2	11	0.6
FSIP1	10	0.6
KIAA0319L	9	0.5
MKL2c	9	0.5
MKL2d	9	0.5
Unknown	9	0.5
PABPC1L	8	0.5
RBM15B	8	0.5
CEPT1	7	0.4
STAT5B	7	0.4

Maldarelli et al. Science 2014

Are all HIV Integrations in Patients on ART in Expanded Clones?

- We think it likely that they are, but we sample only about 10^{-6} of the CD4+ T cell population.

- We might be able to figure it out if we knew the underlying distribution of clones, but this is a very difficult problem…

What does the City Look Like?

Oh

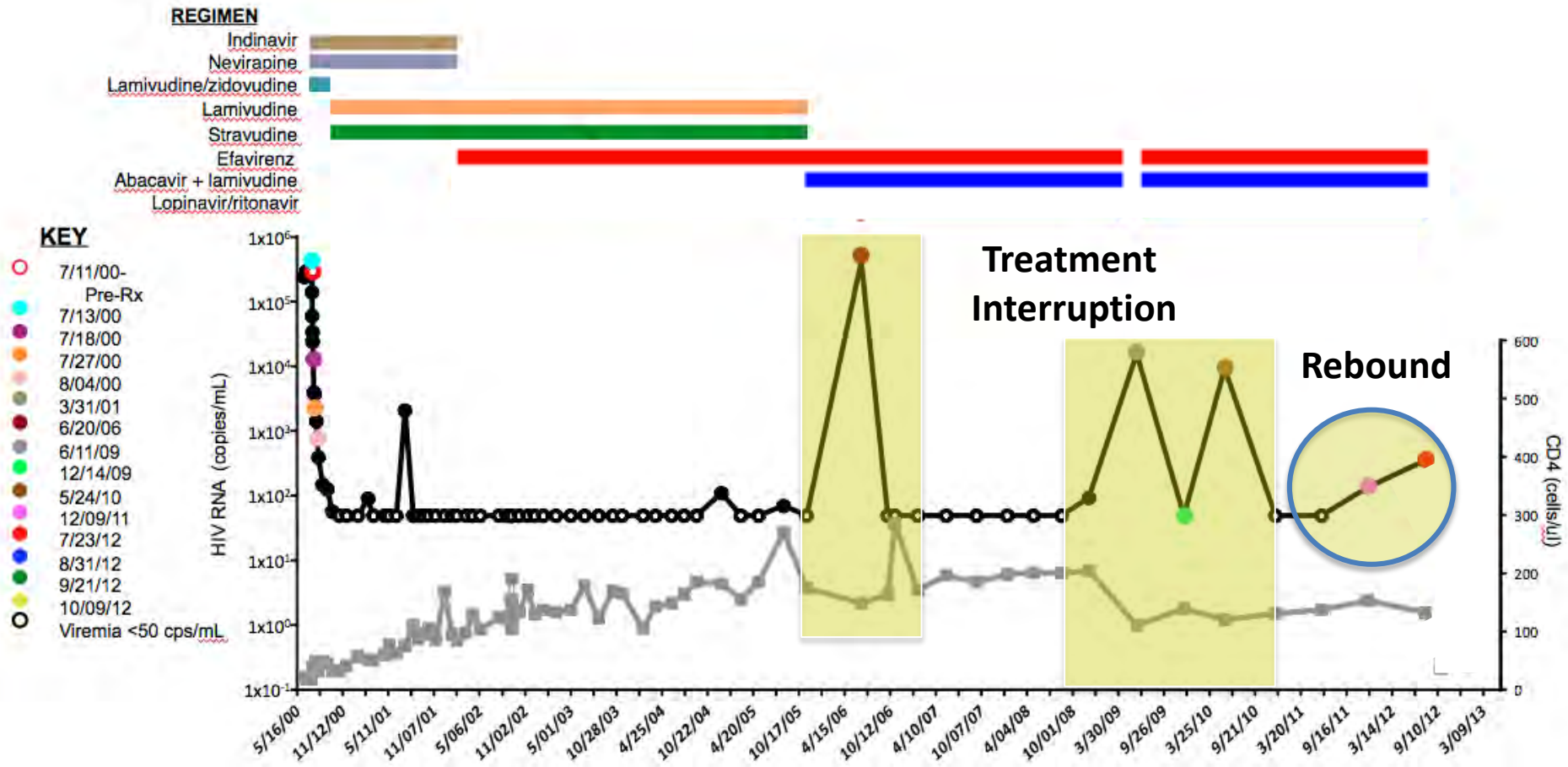
Clonal Expansion of Infected Cells can Arise in Several Ways

- Direct effects of the integrated provirus on cell multiplication or survival
- Chance outgrowth of a memory cell during homeostatic replacement
- Antigen driven expansion during an active immune response

The latter two effects have nothing to do with the provirus, but it serves to mark all descendants of an originally infected cell.

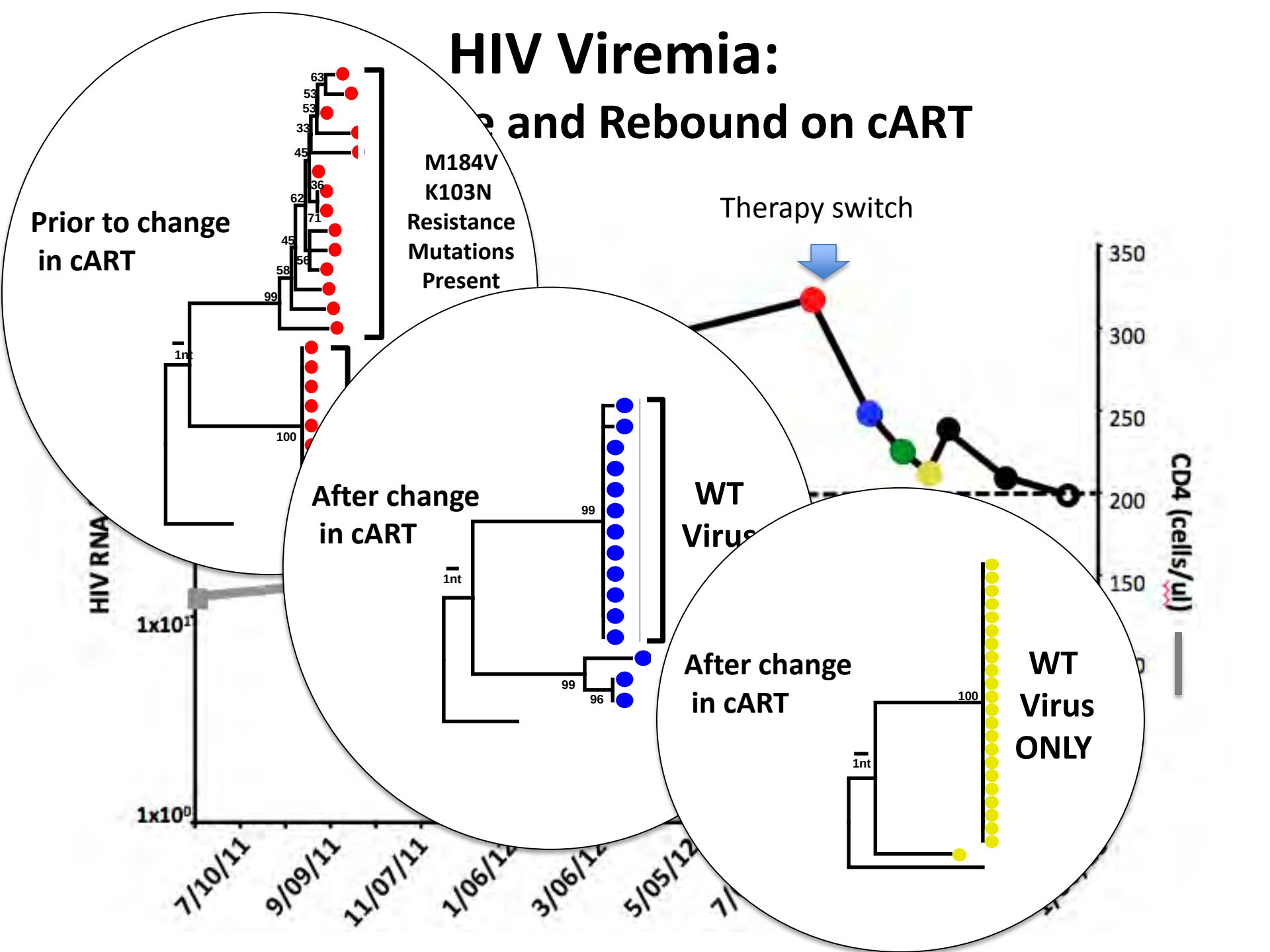
Case Report: Patient 1:

Persistence and Rebound of HIV Viremia on cART

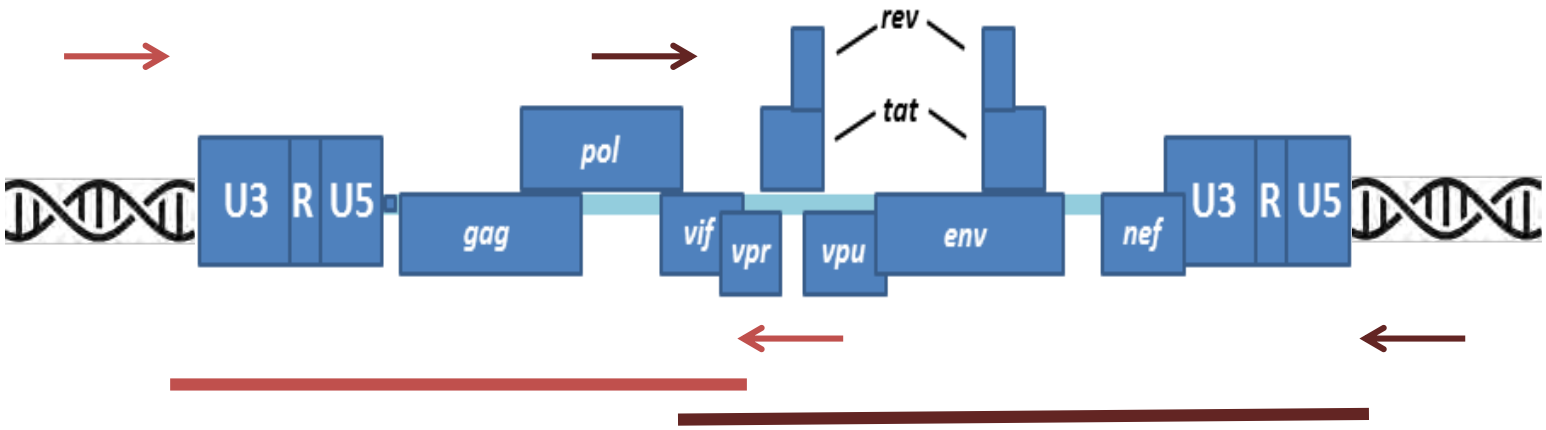


HIV Viremia:

e and Rebound on cART



AMBI-1 Full Provirus is Intact



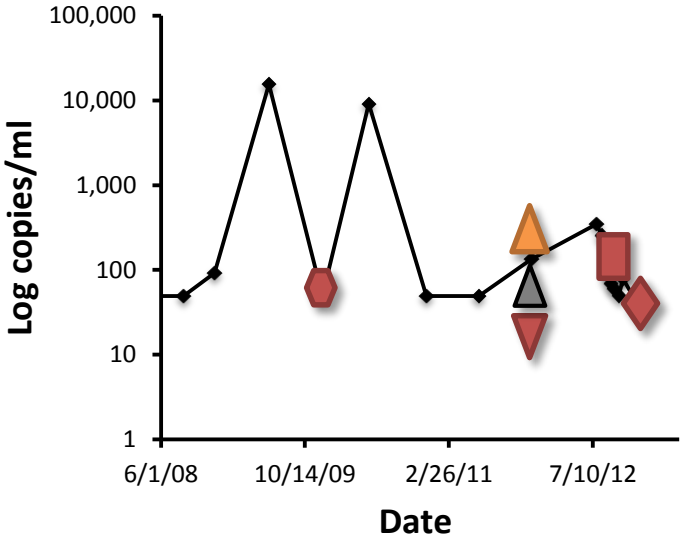
Amplify in overlapping fragments and sequence

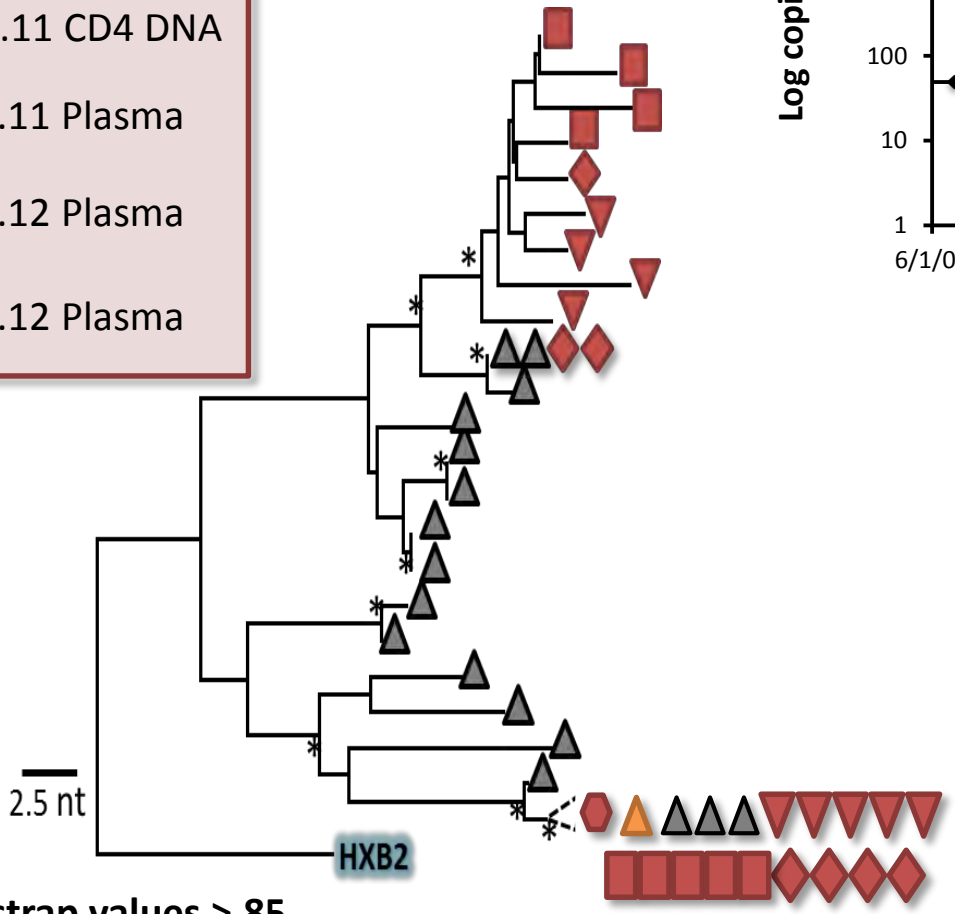
Encodes
Infectious virus!

The Predominant Virus in Plasma is Produced by the AMBI-1 Provirus

P6-RT Sequences

- AMBI-1 provirus
- HIV RNA 12.14.09 Plasma
- HIV DNA 12.09.11 CD4 DNA
- HIV RNA 12.09.11 Plasma
- HIV RNA 07.23.12 Plasma
- HIV RNA 08.31.12 Plasma





Predominant Plasma Clone (PPC)

*Bootstrap values >.85

A Highly Expanded HIV Clone Carries an Infectious Provirus

This is the first known case where we could identify and characterize a clone of latently-infected cells responsible for infectious virus in blood

Only a small fraction of cells express virus RNA (not shown).
What is the epigenetic state of this provirus?
(We don't know yet. It's not DNA methylation)

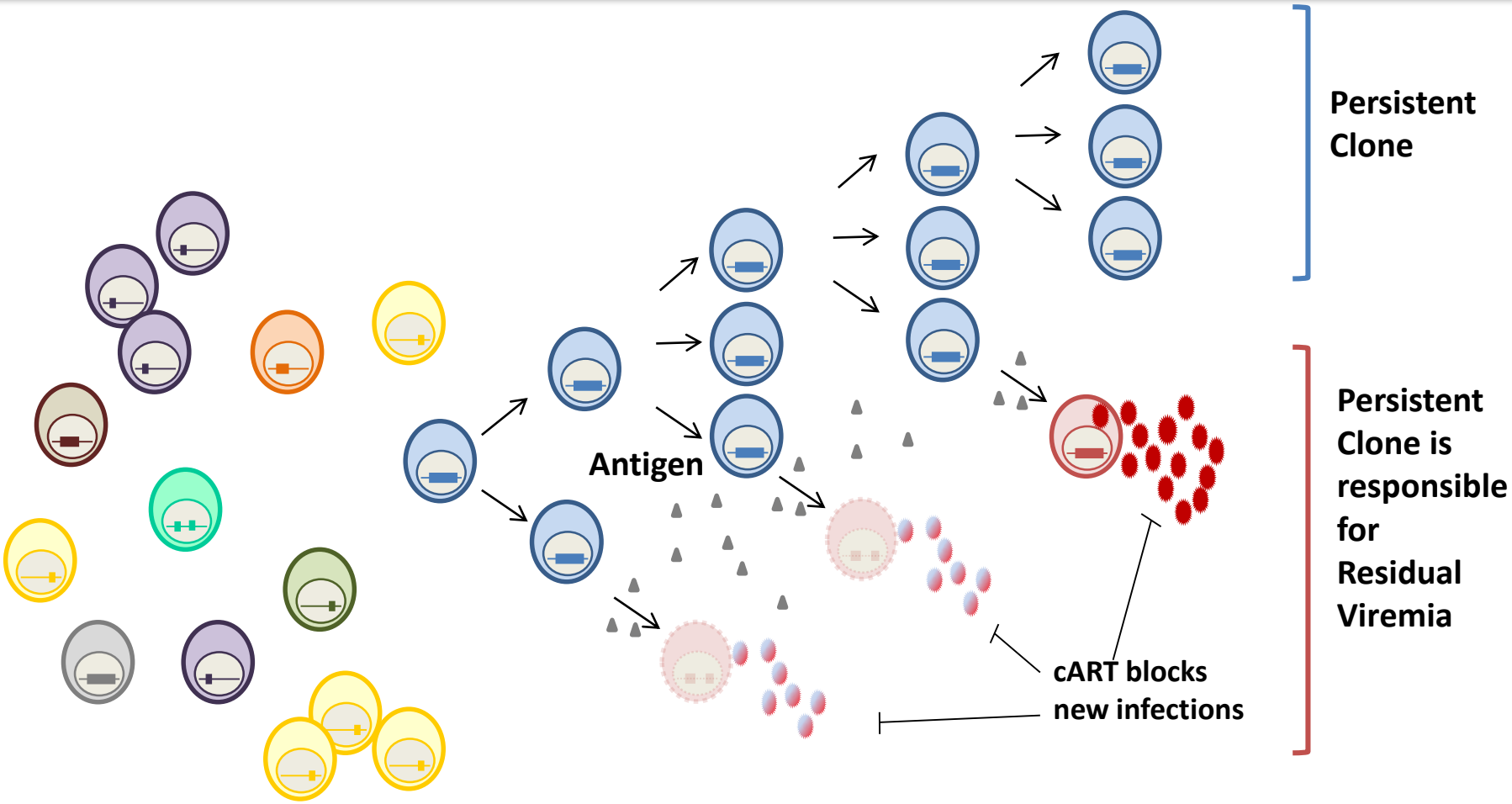
What is driving clonal expansion?
(We know a little bit)

Cells infected with an intact provirus can expand and produce infectious HIV

Despite the potential for viral cytopathic effect and immune-mediated killing of expressing cells, cell clones can both expand and harbor intact proviruses that produce infectious virus over time.

Ca 1% of cells with the AMBI-1 provirus express small amounts of RNA at any one time.

(M. Kearney)



Summary

- We found no evidence for either ongoing replication or compartmentalization of HIV in blood or lymph nodes during suppressive ART, implying that infection is primarily maintained as long-lived cells infected prior to ART.
- Integration site may influence persistence and clonal expansion and therefore, the virus that rebounds
- Expanded clones CAN contain infectious proviruses, and are almost certainly the source of rebound virus when therapy is stopped.
- Curing HIV infection will require completely new strategies for eliminating or otherwise dealing with this expanding persisting reservoir, which must be very large, and is highly variable from one patient to the next.

Acknowledgments

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NIH Bench to Bedside Program

NCI Contract No. HHSN261200800001E

And the Patient Participants!